Supplemental Material

Deficiencies in lamin B1 and lamin B2 cause neurodevelopmental defects and distinct nuclear shape abnormalities in neurons

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Running Head: B-type lamins and brain development

Legends of Supplementary Figures

Figure S1. Expression of Reelin at E12.5–17.5 in the absence of lamin B1. Frozen sections of wild-type (WT) and *Lmnb1*$^{\Delta/\Delta}$ embryos were stained with an antibody against Reelin (red); DNA was counterstained with DAPI (blue). Reelin-positive cells (examples indicated by arrows) were detected in both WT and lamin B1–deficient brains at all stages. Scale bar, 50 μm.

Figure S2. Neuronal nuclear abnormalities in the setting of lamin B2 deficiency. (*A*) Boxplot analysis of the length of nuclei in WT and *Lmnb2*$^{-/-}$ neurons. Nuclear length (μm) was measured on confocal images of brain sections stained with an antibody against lamin B1. Individual boxes show the statistics for cell populations for an individual embryo (with $n = 3$ WT embryos and $n = 4$ *Lmnb2*$^{-/-}$ embryos); limits of each box mark the 25% and 75% percentiles and the middle line, the median; the "whiskers" indicate the range of values; and asterisks, the outlier values. The table shows the number of nuclei (n) measured for each embryo and the upper and lower limits of the 95% confidence interval (CI), expressed in μm. Comparison of the mean values of nuclear length between WT and *Lmnb2*$^{-/-}$ embryos with a two-tailed Student t-test yielded a P-value < 0.0001. (*B*) Elongated nuclei and distant centrosomes in neurons from *Lmnb2*$^{-/-}$ embryos. Neuronal progenitors were isolated from the cortex of WT or *Lmnb2*$^{-/-}$ embryos at E13.5 and cultured in differentiation medium for four days. Cells were stained with antibodies against lamin B1 (red), pericentrin (green), and neuron-specific β-tubulin III (TubIII, magenta). DNA was stained with DAPI (blue). Arrowheads indicate stretched nuclei and arrows indicate the centrosome. Scale bar, 50 μm.

Figure S3. Nuclear shape abnormalities and asymmetric distribution of lamin B2 in neurons from *Lmnb1*$^{\Delta/\Delta}$ embryos. Neuronal progenitors were isolated from cortical explants from WT or *Lmnb1*$^{\Delta/\Delta}$ embryos at E13.5 and cultured in differentiation medium for four days. Cells were stained for lamin B2 (red) and Lap2β (green), and images were recorded at low (*A*) and high magnification (*B*). Examples of cells with nuclear blebs, or with an irregular distribution of lamin B2, are noted with arrowheads. Scale bar, 20 μm.

Figure S4. Forebrain-specific inactivation of *Lmnb1* and *Lmnb2*. (*A*) β-Galactosidase staining on the brain from an adult mouse carrying an *Emx1-Cre* transgene and a *Cre*-activated *ROSA26-lacZ* reporter gene. (*B*) Immunostaining on brain sections from E15.5 *Emx1-Cre Lmnb1*$^{fl/+}$ and *Emx1-Cre Lmnb1*$^{fl/fl}$ embryos with an antibody against lamin B1, showing *Lmnb1* inactivation in the forebrain of the *Emx1-Cre Lmnb1*$^{fl/fl}$ embryo. Ctx, cortex; cer, cerebellum; mb, midbrain; po, pons; str, striatum; th, thalamus. (*C*) Immunostaining of the forebrain of E15.5 *Emx1-Cre Lmnb1*$^{fl/+}$ and *Emx1-Cre Lmnb1*$^{fl/fl}$ embryos with an antibody against lamin B1 (green), and immunostaining of sections from E15.5 *Emx1-Cre Lmnb2*$^{fl/+}$ and *Emx1-Cre Lmnb2*$^{fl/fl}$ embryos with an antibody against lamin B2 (red), showing the presence of cells lacking either lamin B1 or lamin B2, respectively. DNA was stained with DAPI (blue). *D*) Immunostaining of the

forebrain of E17.5 *Lmnb1*$^{fl/fl}$ *Lmnb2*$^{fl/+}$ (control) and *Emx1-Cre Lmnb1*$^{fl/fl}$ *Lmn2*$^{fl/fl}$ embryos with antibody against lamin B1 (green), and lamin B2 (red), identifying cells lacking both lamins B1 and B2. DNA was stained with DAPI (blue) Scale bar in *A*, 2.5 mm; *B*, 1 mm; *C-D*, 50 μm.

Figure S5. Nuclear shape abnormalities in the forebrain of E15.5 *Emx1-Cre Lmnb1*$^{fl/fl}$ and *Emx1-Cre Lmnb2*$^{fl/fl}$ embryos. (*A*) Confocal images of forebrain sections of E15.5 *Emx1-Cre Lmnb1*$^{fl/+}$ and *Emx1-Cre Lmnb1*$^{fl/fl}$ embryos stained with antibodies against Lap2β (red) and lamin B1 (green), or lamin B2 (red) and DAPI (blue). Arrowheads indicate cell nuclei with blebs (top) or an asymmetric distribution of lamin B2 (bottom). (*B*) Confocal immunofluorescence images of sections from the forebrain of *Emx1-Cre Lmnb2*$^{fl/+}$ and *Emx1-Cre Lmnb2*$^{fl/fl}$ E15.5 embryos stained with antibodies against Lap2β (green) and lamin B2 (red), or lamin B1 (green) and DAPI (blue). Arrowheads indicate elongated cell nuclei. Scale bars, 20 μm.

Figure S6. Differential *Lmna* expression in the skin and brain of mouse embryos and adult mice. (*A*) Comparison of *Lmnb1, Lmnb2,* and *Lmna* expression patterns in the skin at E15.5 and E19.5, as judged by β-galactosidase staining. Frozen sections from *Lmnb1*$^{+/\Delta}$, *Lmnb2*$^{+/-}$, and *Lmna*$^{lacZ/+}$ embryos were stained for β-galactosidase activity and then counterstained with eosin. Arrows indicate scattered β-galactosidase-positive cells in the skin of the E19.5 *Lmnb2*$^{+/-}$ embryo. (*B*) Immunostaining of E17.5 embryosof the following genotypes: control (*Lmnb1*$^{fl/fl}$ *Lmnb2*$^{fl/+}$), embryo lacking lamin B1 in the forebrain (*Emx1-Cre Lmnb1*$^{fl/fl}$ *Lmnb2*$^{fl/+}$); embryo lacking lamin B2 in the forebrain (*Emx1-Cre Lmnb1*$^{fl/+}$ *Lmnb2*$^{fl/fl}$); and double knockout lacking both lamin B1 and lamin B2 in the forebrain (*Emx1-Cre Lmnb1*$^{fl/fl}$ *Lmnb2*$^{fl/fl}$) with antibodies against lamins A/C (red) and the layer VI marker TBR1 (green). Note the strong signal for lamins A and C in the skin (sk) and mesenchyme of the skull (m) compared with absent signals in the cortical plate (cp). Dotted lines mark the border between the cortex and mesenchyme. (*C*) β-galactosidase staining of brain of adult *Lmnb1*$^{+/\Delta}$, *Lmnb2*$^{+/-}$, and *Lmna*$^{lacZ/+}$ mice. Brains were cut along the sagittal plane to expose internal structures prior to staining. (*D–E*) Immunostaining of the cortex and hippocampus in 1-month old control (*Emx1-Cre Lmnb1*$^{fl/+}$) and *Emx1-Cre Lmnb1*$^{fl/fl}$ mice stained with antibodies against lamin B1 (green), lamin B2 (red), and lamins A/C (magenta). (D) Confocal images of forebrain sections in adult mice. All cortical neurons expressed lamins A/C. Note the normal distribution of lamin B2 (red) in lamin B1–deficient cells (arrowheads). (E) Confocal images of the hippocampus. Dotted line marks the outer border of the dentate gyrus in the *Emx1-Cre Lmnb1*$^{fl/fl}$ mouse. Unlike the adult cortex, the level of lamin A/C expression in the hippocampus was less uniform and less intense; thus, the lamin B1–deficient cells (red) along the outer edge of the dentate gyrus expressed lower levels of lamins A/C. Interestingly, these lamin B1–deficient cells exhibited an abnormal distribution of lamin B2 at the nuclear rim (also see Fig 6B). Scale bars: panel A top, 50 μm; *bottom*, 100 μm; panel *B*, 100 μm; panel *C*, 2.5mm; panels *D–E*, 50 μm.

Table S1. List of primary antibodies with dilutions used for immunocytochemistry (ICC) and immunohistochemistry (IHC).

Antigen	**Antibody**	**Species**	**Company**	**ICC**	**IHC**
BrdU [BU1/75]	Monoclonal	Rat	Abcam		1:200
Caspase-3, active [C92-605]	Monoclonal	Rabbit	BD Biosciences		1:200
CDP/Cux1 [M-222]	Polyclonal	Rabbit	Santa Cruz Biotech.		1:100
Chondroitin Sulfate [CS-56]	Monoclonal	Mouse	Sigma		1:400
Ctip2 [25B6]	Monoclonal	Rat	Abcam		1:500
Ki67 [MM1]	Monoclonal	Mouse	Novo Castra, Leica		1:100
L1	Monoclonal	Rat	Chemicon, Millipore		1:200
Lamin B1 [M-20]	Polyclonal	Goat	Santa Cruz Biotech.	1:400	1:400
Lamin B2 [E-3]	Monoclonal	Mouse	Zymed, Invitrogen	1:50	1:200
Lap2β [27]	Monoclonal	Mouse	BD Transduction Lab	1:400	1:400
NeuN	Monoclonal	Mouse	Millipore		1:500
Otx1	Polyclonal	Rabbit	Abcam		1:100
Pericentrin	Polyclonal	Rabbit	Abcam	1:1000	
Reelin [G10]	Monoclonal	Mouse	Chemicon, Millipore		1:500
Sox2	Polyclonal	Goat	Santa Cruz Biotech.		1:100
TBR1	Polyclonal	Rabbit	Abcam		1:100
TBR2/Eomes	Polyclonal	Rabbit	Abcam		1:500
βIII-Tubulin [TU-20]	Monoclonal	Mouse	Abcam	1:1000	

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Figure S1

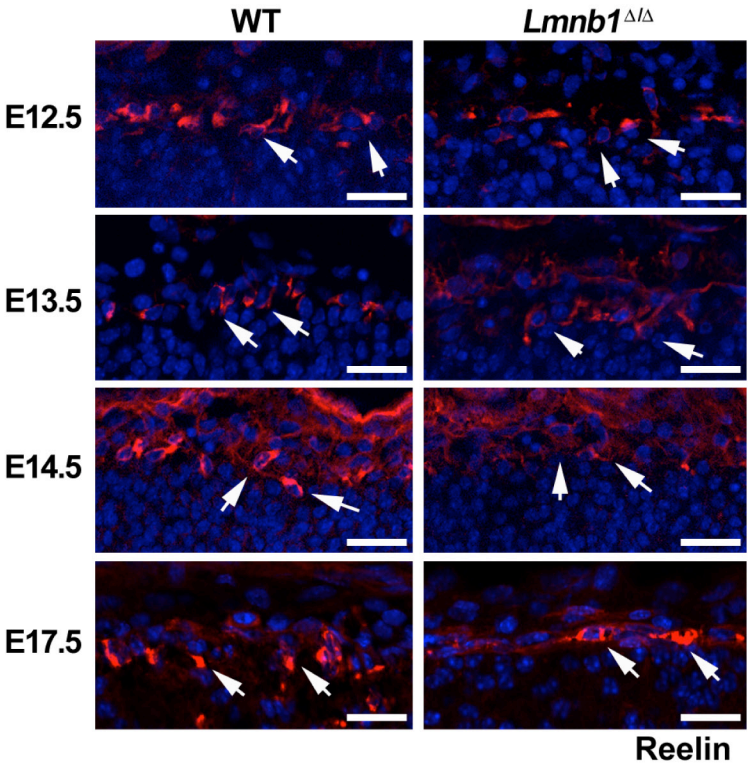

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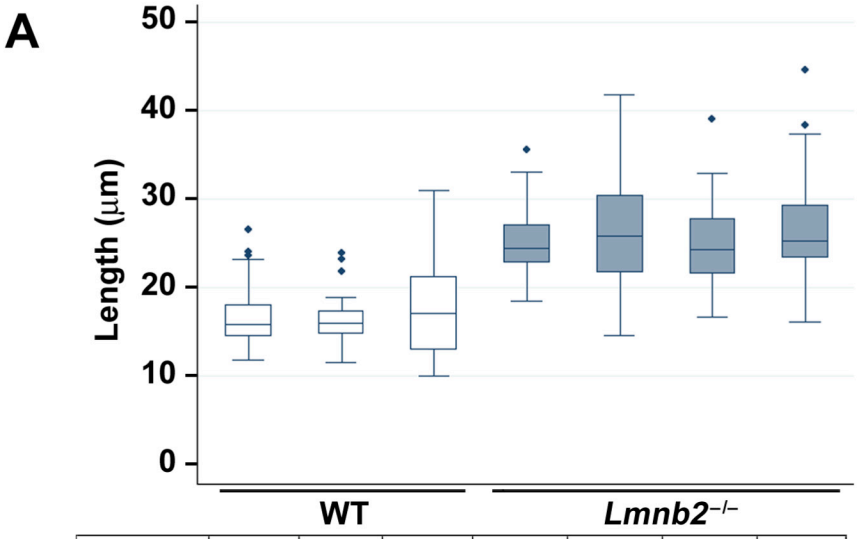
Figure S2



n	68	50	63	20	36	27	76
95% CI lower	15.72	15.54	16.27	23.34	24.13	22.91	25.03
95% CI upper	17.23	16.84	18.76	27.25	28.11	26.60	27.26

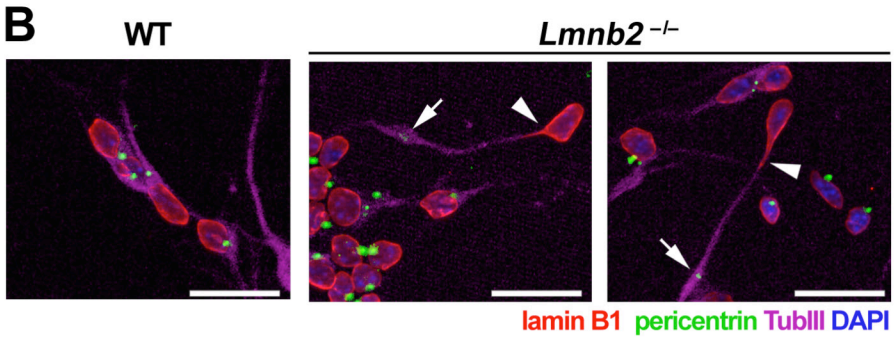


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Figure S3

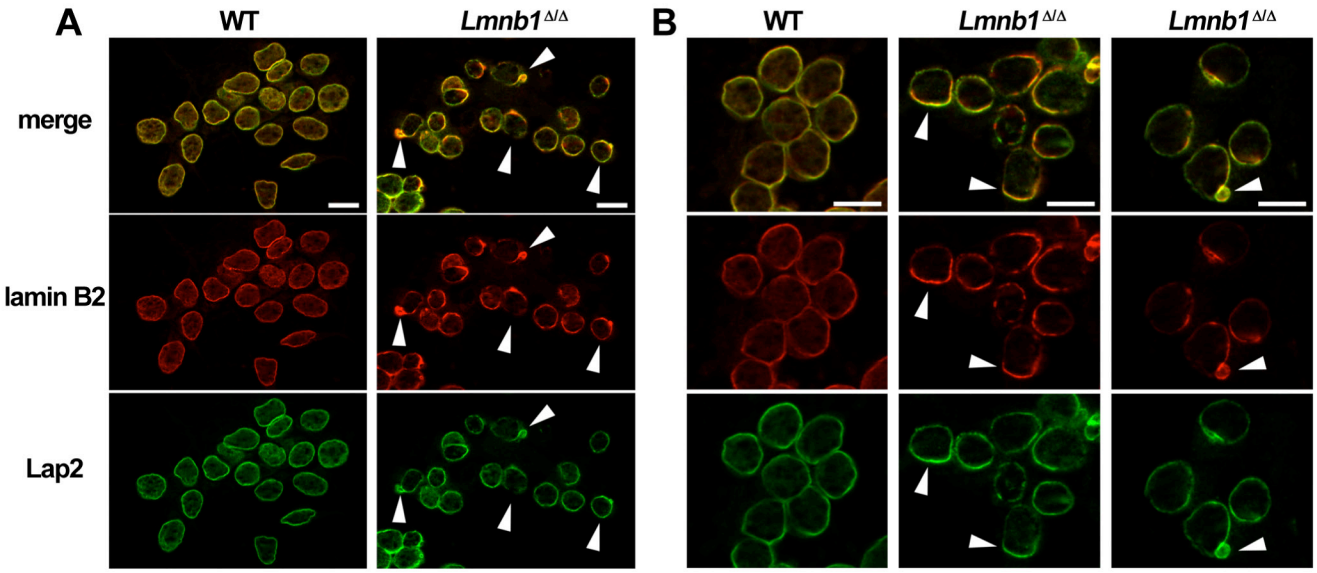

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Figure S4

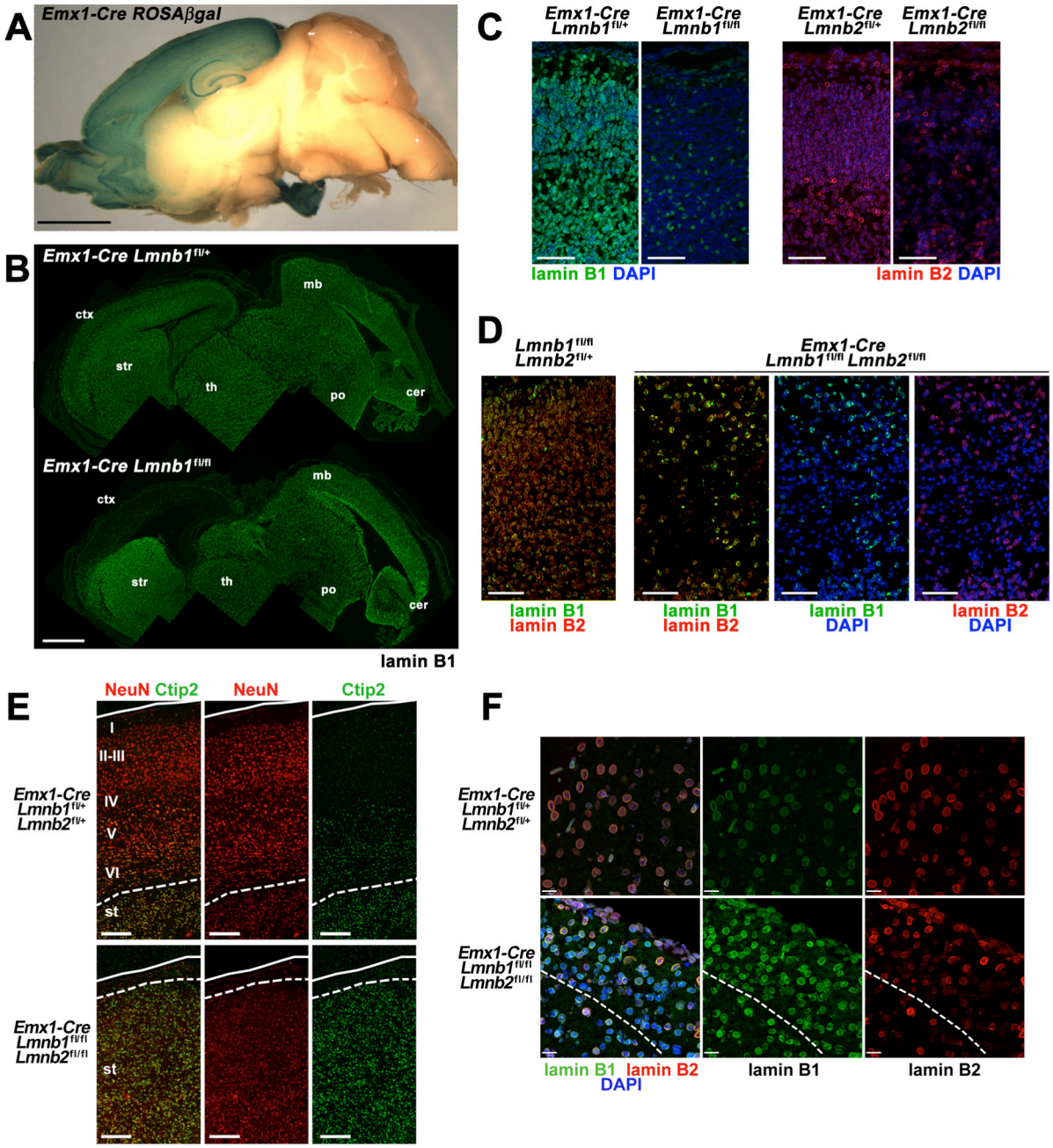

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Figure S5

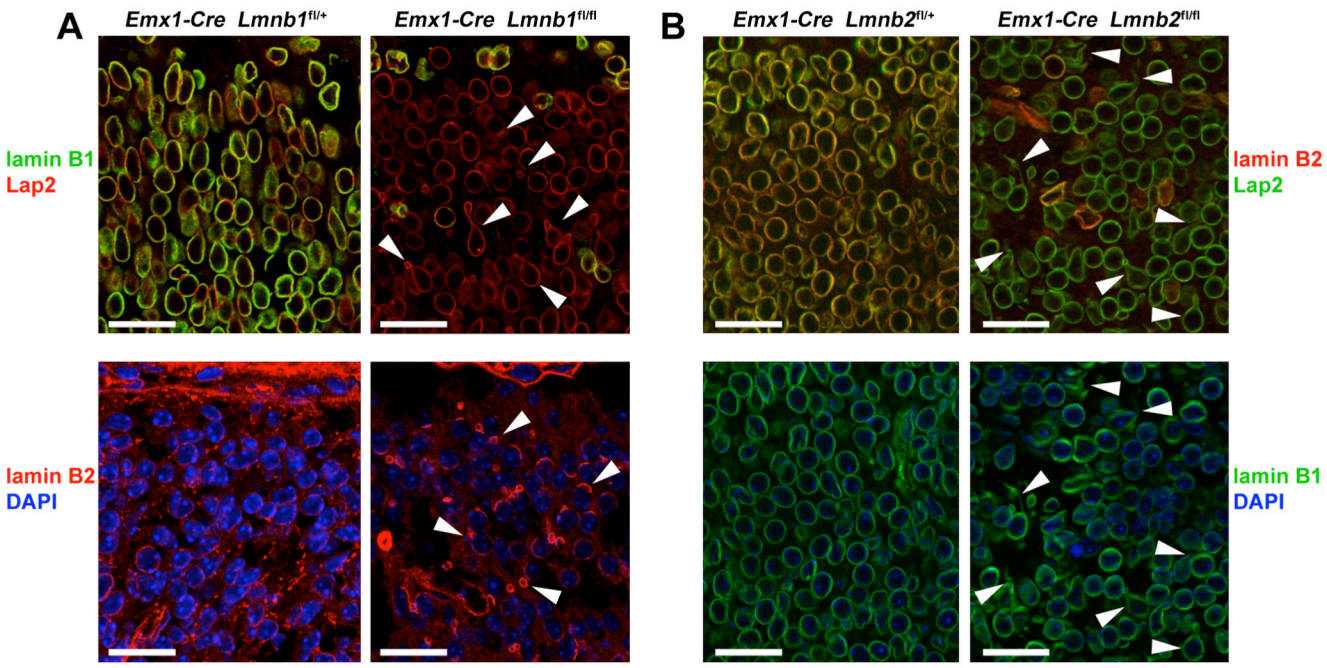

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Figure S6